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iPTH values during hemodialysis: Role of ionized Ca, dialysis membranes and iPTH assays

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iPTH values during hemodialysis: Role of ionized Ca, dialysis membranes and iPTH assays. The evolution of serum iPTH concentration during hemodialysis was studied in eight patients who were dialyzed with cuprophane (Cu) and polyacrylonitrile membranes (PAN) during two four-hour sessions. Ca^{++} concentration in the dialysate was 1.37 mM/liter. iPTH was measured with an intact hormone immunoradiometric assay (I), with two late (L_1, L_2) and one mid (M) carboxylterminal immunoassays at the beginning and end of hemodialysis, from the arterial and venous sides of the extracorporeal unit. Results are means ± SD. Serum Ca^{++} increased during dialysis with Cu (1.26 ± 0.08 vs. 1.33 ± 0.03 mmol/liter, $P < 0.05$), without any change in the concentration of iPTH measured with L_1, L_2 or M, but with a 50% decrease in iPTH measured with I (21.8 ± 19.2 vs. 10.3 ± 9.0 pmol/liter, $P < 0.05$). Serum Ca^{++} increased similarly with PAN (1.25 ± 0.10 vs. 1.34 ± 0.04 mmol/liter, $P < 0.01$), but there was a greater than 50% decrease in iPTH concentration measurements for all four assays (I: 17.2 ± 17 vs. 7.6 ± 8.3 pmol/liter, $P < 0.05$; L_1: 92 ± 75 vs. 36 ± 32 pmol/liter, $P < 0.05$; L_2: 312 ± 289 vs. 126 ± 128 pmol/liter, $P < 0.01$; M: 926 ± 1074 vs. 373 ± 422 pmol/liter, $P < 0.05$). iPTH clearance was minimal through Cu and significantly higher through PAN with I (124 ± 33 ml/min, $P < 0.005$), L_1 (79 ± 17 ml/min, $P < 0.0005$), L_2 (83 ± 19 ml/min, $P < 0.0005$) and M (52 ± 25 ml/min, $P < 0.05$); since intact hormone was cleared more readily than large fragments and the latter more readily than small fragments, hydrophobic adherence to the PAN was first postulated and then demonstrated during in vitro dialysis of ^{131}I-bPTH(1-84) and ^{125}I-bPTH(41-84). Thus, increasing serum Ca^{++} concentration during hemodialysis reduced the circulating level of intact PTH by half without influencing the concentration of carboxylterminal fragments. The concentration of the latter could be reduced by more than 50%, and that of intact hormone by a further 25% by selection of a PAN which permitted extracorporeal clearance of iPTH. These results suggest that the predialysis concentration of iPTH, and mostly of intact iPTH, is best to evaluate the parathyroid status of hemodialyzed patients, since various factors will influence iPTH concentration during and immediately after hemodialysis.

Once it had been demonstrated that i.v. calcium infusion could decrease serum immunoreactive parathyroid hormone (iPTH) in patients with chronic renal failure [1, 2], various attempts were made to reduce secondary hyperparathyroidism in these patients by increasing the calcium concentration of the dialysate during hemodialysis [3–8]. Although it was sometimes possible to significantly reduce serum iPTH during hemodialysis by this means [2, 5, 8], no long-term effect could be demonstrated on the parathyroid function [3, 4, 6, 7]. Initially, dialysis membrane permeability to iPTH or its fragments was not evaluated to explain the decrease in serum iPTH during hemodialysis [2, 5, 8]. Later studies were able to demonstrate some permeability of dialysis membranes to iPTH fragments [9–14] and occasionally to intact PTH(1-84) [12]. If the decrease in fragments could readily be explained by membrane permeability and clearance, most authors felt that the decrease in intact hormone was related to the increased serum concentration of calcium in these patients [12, 13]. Since dialysis membranes of much greater permeability are now currently available for hemodialysis, we were interested in seeing whether we could significantly reduce all molecular forms of serum iPTH in chronic renal failure during hemodialysis, irrespective of changes in serum calcium. This study thus compares the influence of two dialysis membranes of different permeability on serum iPTH concentration during hemodialysis.

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Methods

Subjects

Eight subjects suffering from chronic renal failure were recruited from patients regularly attending our unit for hemodialysis. They were aged from 20 to 69 and had been on hemodialysis from 1 to 57 months. Glomerulonephritis was the diagnosis in four patients, nephrosclerosis in two, focal sclerosis in one and chronic pyelonephritis in the other. All were treated with aluminum hydroxide but none had had prior parathyroid surgery nor a renal transplant. Informed consent was obtained from the eight subjects.

Experimental protocol

In vivo dialysis. For each patient, iPTH diffusion across the dialysis membrane was studied at the beginning and end of two consecutive dialysis sessions. Half of the patients were first dialyzed with a cuprophane membrane (Discap 160, Hospal), and the other half with a polyacrylonitrile membrane (Filtral, Hospal); the procedure was reversed for the second session. These two hollow fiber membranes have similar surfaces areas (Discap 160, 1.22 m^2; Filtral, 1.15 m^2) and urea clearance, but the polyacrylonitrile has a greater permeability to middle molecules. Blood was sampled at the beginning and end of each dialysis session from both the arterial and the venous puncture sites of the extracorporeal circuit. For purposes of the study,

both dialysis sessions were standardized at four hours and an acetate dialysate was used containing 1.37 mmol/liter ionized Ca^{++}, delivered at 500 ml min^{-1} by a single pass delivery system (Monitral, Hospal). Blood flow varied from 200 to 250 ml min^{-1}, but was maintained at the same rate for the two sessions for any given patient. The ultrafiltration rate was adjusted according to patient needs, but was turned off five minutes before blood sampling.

In vitro dialysis. In vitro dialysis of ^{131}I-bPTH(1-84) and of ^{125}I-bPTH(41-84) was next performed. Iodination of bPTH(1-84) with $Na^{125}I$ or $Na^{131}I$ (Amersham Canada Ltd., Oakville, Ontario, Canada), generation of ^{125}I-bPTH(41-84) by incubating ^{125}I-bPTH(1-84) with rat kidney membranes, and purification of both iodinated preparations by gel chromatography on Bio-Gel P-100 (Bio-Rad Laboratories, Richmond, California, USA) were accomplished as previously described [15, 16]. ^{125}I-bPTH(41-84), 42.6×10^6 cpm, and ^{131}I-bPTH(1-84), 18.6×10^6 cpm, were added to 1250 ml of serum coming from the plasmapheresis of one patient; this solution was further diluted to five liters with NaCl 0.9%. This solution was then pumped at a flow of 75 ml/min alternatively into three Discap 160 and three Filtral filters. In this later case, filtration pressure was adjusted at 50, 100, 200, and 275 mm of Hg for periods of 10, 5, 5, and 5 minutes, respectively. For the former cuprophane membrane, the filtration pressure was adjusted at 200 mm of Hg for 15 minutes and then 275 for 10 minutes. The ultrafiltrate and the solution coming out of the filter were continuously returned to the original five liter solution. Samples of the solution coming in and out of the filter, as well as from the ultrafiltrate, were obtained simultaneously from each filter at each of the filtration pressures. Total protein concentration was measured by a standard automated method and radioactivity estimated in a dual energy Y-counter (Gamma 8000, Beckman Instruments, Fullerton, California, USA). Ultrafiltrate flow was estimated indirectly from the difference observed in total protein concentration between the entrance and the exit of the filter.

The amount of ^{131}I-bPTH(1-84) and of ^{125}I-bPTH(41-84) which adhered to each type of filter was also evaluated. The plastic container of each filter was sawn and samples of fibers coming from each third of a filter were obtained, weighed, and counted in a dual energy Y-counter. Results for each filter represent a mean of nine samples, three in each third of a filter; they are expressed in cpm/g of fibers. This procedure was necessary because the amount of ^{131}I or ^{125}I radioactivity decreased progressively from entrance to exit.

Laboratory methods

Serum ionized calcium was measured within one hour of sampling by a calcium sensitive electrode (ICA-1, Radiometer, Copenhagen). BUN, creatinine, total proteins, albumin, and total calcium were measured by standard colorimetric methods adapted to multianalyzer analysis. Serum parathyroid hormone was measured by means of four different PTH immunoassays. In each case, all the samples to be measured were analyzed within a single assay. The first assay was a commercial radioimmunometric assay for intact human PTH(1-84) (Allegro Intact PTH, Nichols Institute, San Juan Capistrano, California, USA); the intra-assay coefficient of variation on duplicate was 3.1%. The second assay was a late carboxylterminal assay developed in our laboratory [15]; antiserum C-52 was presaturated with synthetic hPTH(44-68) to eliminate the low-affinity mid carboxylterminal component and used at 1/50,000 final dilution with ^{125}I-[tyr^{52}]hPTH(52-84) as tracer and synthetic hPTH(39-84) as standard. Under these conditions, the assay reacted mainly with large carboxylterminal fragments of the hormone [17]. The intra-assay coefficient of variation was 3.3%. The third assay was also a late carboxylterminal assay developed in our laboratory [17]; antiserum C-97 was used at 1/60,000 final dilution with ^{125}I-[tyr^{52}]hPTH(52-84) as tracer and synthetic hPTH(39-84) as standard. Under these conditions, the assay reacted mainly with large carboxylterminal fragments, but also with smaller fragments not recognized by antiserum C-52 [17]. The intra-assay coefficient of variation was 7.4%. The last assay was a mid carboxylterminal assay developed by L.E. Mallette [18, 19]; antiserum G-5 was used at 1/10,000 final dilution with ^{125}I-[tyr^{43}]hPTH(43-68) as tracer and hPTH(39-68) as standard. This assay reacted with the molecular forms of iPTH recognized by the other two late carboxylterminal assays and also with large and small mid carboxylterminal fragments. The intra-assay coefficient of variation was 5.2%. Other conditions for the last three assays are as previously described [17, 19]. Assays C-52 and C-97 were performed first, followed by the intact hormone assay and, finally, G-5. Some samples were unavailable for the last two assays, decreasing the final number of patients for the clearance studies. To obviate for the hemoconcentration effect observed with both filters during hemodialysis, iPTH values were corrected to the total protein concentration observed in each patient at the beginning of hemodialysis.

Samples from a single patient were analyzed by gel chromatography. They were obtained during the early stages of each dialysis session from both sides of the extracorporeal dialysis unit. In each case, 2 ml plasma was fractionated on a 1.2×100 cm column of Bio-Gel P-100 (Bio-Rad Laboratories), equilibrated and eluted with a 0.1 M ammonium acetate buffer, pH 4.6, containing 1% bovine serum albumin. The 1 ml fractions obtained were lyophilized and reconstituted with the iPTH assay buffer; appropriate dilutions were then assayed with the three carboxylterminal antisera. In these studies, standard hPTH(1-84) was used to analyze the gel chromatography profile region corresponding to hPTH(1-84), while standard hPTH(39-84) was utilized to analyze that corresponding to carboxylterminal fragments [20]. iPTH recovery was better than 80% for each of the four profiles and the three different assays.

Statistical analysis

Paired *t*-tests were used for comparison. Results are expressed as means ± SD.

Results

The influence of each filter and a dialysate ionized calcium concentration of 1.37 mmol/liter on various biochemical parameters following four hours of hemodialysis is illustrated at Table 1. The decrease in BUN and creatinine observed over the dialysis period was similar with both filters. Although the increase in pH was slightly higher with the polyacrylonitrile filter, the post-dialysis pH was similar in both groups mainly because pH was initially slightly lower with the polyacrylonitrile membrane prior to dialysis. The increase in total proteins and albumin was also significantly higher with this latter mem-

Table 1. Biochemical data in patients before and after 4 hours of hemodialysis with two different membranes at a dialysate Ca^{++} concentration of 1.37 mM/liter

Parameters measured	Filter					
	Cuprophane			Polyacrylonitrile		
	Before	After	Δ	Before	After	Δ
BUN *mmol/liter*	25.1 ± 5.7	10.4 ± 3.6[c]	−14.6 ± 4.7	29.3 ± 7.7[d]	13.4 ± 4.2[cd]	−16.0 ± 4.4
Creatinine *μmol/liter*	913 ± 258	439 ± 112[c]	−474 ± 194	1037 ± 301[d]	518 ± 157[cd]	−519 ± 154
pH	7.33 ± 0.05	7.46 ± 0.04[c]	0.13 ± 0.03	7.30 ± 0.06[d]	7.46 ± 0.04[c]	0.17 ± 0.04[d]
Proteins *g/liter*	65.0 ± 5.0	71.0 ± 8.0[a]	6.0 ± 6.7	64.6 ± 3.9	75.0 ± 9.1[bd]	10.4 ± 7.5[d]
Albumin *g/liter*	38.4 ± 3.3	40.6 ± 6.4	2.2 ± 4.2	38.4 ± 3.0	43.8 ± 6.6[be]	5.4 ± 4.2[d]
Ca_t *mmol/liter*	2.01 ± 0.25	2.44 ± 0.32[b]	0.43 ± 0.37	1.95 ± 0.27[d]	2.69 ± 0.13[cd]	0.75 ± 0.24[e]
Ca^{++} *mmol/liter*	1.26 ± 0.08	1.33 ± 0.03[a]	0.07 ± 0.07	1.25 ± 0.10	1.34 ± 0.04[b]	0.09 ± 0.07[d]
PTH-IRMA *pmol/liter*	21.8 ± 19.2	10.3 ± 9.0[a]	−11.5 ± 12.0	17.2 ± 17.0	7.6 ± 8.3[ad]	−9.6 ± 10.8
PTH-Late C-52 *pmol/liter*	105 ± 93	94 ± 108	−11 ± 39	92 ± 75	36 ± 32[ad]	−55 ± 47[d]
PTH-Late C-97 *pmol/liter*	354 ± 375	343 ± 386	−11 ± 59	312 ± 289	126 ± 128[ad]	−186 ± 164[d]
PTH-Mid G-5 *pmol/liter*	901 ± 1049	701 ± 732	−188 ± 330	926 ± 1074	373 ± 422[adg]	−625 ± 737[d]

Results are means ± SD of 8 patients.
Before vs. after [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$
Cuprophane vs. polyacrylonitrile [d] $P < 0.05$, [e] $P < 0.01$, [f] $P < 0.001$, [g] $N = 7$

brane, partially explaining the more pronounced increase in total blood calcium. Post-dialysis ionized serum calcium was higher, but similar, with both membranes. Despite the increase in ionized calcium, serum iPTH measured with both late carboxylterminal assays and with the mid carboxylterminal assay did not decrease during hemodialysis with the cuprophane membrane; only intact iPTH decreased by about 50%. While there was a similar increase in ionized calcium with the polyacrylonitrile membrane, serum iPTH measurements decreased by more than 50% in each of the four assays during hemodialysis. Final intact iPTH levels were 25% lower ($P < 0.05$) with the polyacrylonitrile filter.

Some of these results are explained by the technical dialysis data on the patients (Table 2). Urea and creatinine clearances were similar with both filters, explaining the similar decreases in BUN and creatinine. Ultrafiltration was adjusted at a slightly higher rate with the polyacrylonitrile membrane, hence the higher concentrations of albumin, total proteins and total calcium observed in this group. There was minimal extracorporeal iPTH clearance with the cuprophane membrane at the beginning or end of hemodialysis using any of the iPTH assays (Table 3). Furthermore, only for the mid assay was there a significant decrease in the value observed between the beginning and end of hemodialysis. On the other hand, with the polyacrylonitrile membrane, iPTH clearances measured by the four iPTH assays were present at the beginning and end of hemodialysis. Values obtained with the intact hormone assay were very near those observed for extracorporeal creatinine clearance at the beginning of hemodialysis (Fig. 1) and significantly less at the end of hemodialysis; iPTH clearances measured by means of the intact hormone assay were also significantly higher than clearances measured using the other three assays, both at the beginning and end of hemodialysis. iPTH clearances measured with the late carboxylterminal assays were about 60% of the intact hormone clearance both at the beginning and the end of hemodialysis, and these values were higher than those observed with the mid carboxylterminal assay. With this latter assay, iPTH clearances were similar at the beginning and at the end of hemodialysis.

This is shown graphically in Figure 1, with the ratio of iPTH clearance/creatinine clearance being illustrated for both filters, at the beginning and end of hemodialysis. With cuprophane, the mean ratio remains low at the beginning (Intact, 0.25 ± 0.46; Late C-52, 0.08 ± 0.19; Late C-97, 0.03 ± 0.24; Mid, 0.19 ±

Table 2. Technical dialysis data in patients

Patient no.	QBI *liter/min*	Filter					
		Cuprophane			Polyacrylonitrile		
		UF *liter/4 hr*	Cl_{Ur} *ml/min*	Cl_{Cr} *ml/min*	UF *liter/4 hr*	Cl_{Ur} *ml/min*	Cl_{Cr} *ml/min*
1	0.23	1.1	171	126	2.6	158	138
2	0.25	3.6	158	130	4.0	169	135
3	0.25	4.2	172	157	4.5	152	133
4	0.24	2.4	151	122	4.0	151	131
5	0.20	2.8	106	98	3.3	154	125
6	0.22	2.0	165	132	2.9	146	119
7	0.25	0.8	164	136	1.0	143	116
8	0.20	3.0	128	109	2.8	117	106
Mean	0.23	2.50	152	126	3.1[a]	149	125
SD	± 0.02	± 1.16	± 23	± 18	± 1.1	± 15	± 11

Abbreviations are: QBI, blood flow; UF, ultrafiltrate; Cl_{Ur}, urea clearance, Cl_{Cr}, creatinine clearance.
[a] Cuprophane vs. polyacrylonitrile: $P < 0.05$

Table 3. Influence of dialysis filter characteristics on iPTH clearance when iPTH is measured with assays of different specificity at the beginning and end of 4 hours of hemodialysis

	Cuprophane		Polyacrylonitrile	
	Beginning	End	Beginning	End
PTH assay	*ml/min*			
1) PTH-IRMA	32 ± 60	−20 ± 37[k]	124 ± 33[b]	90 ± 17[cdk]
2) PTH-Late C-52	10 ± 23	10 ± 27	79 ± 17[cg]	64 ± 18[afk]
3) PTH-Late C-97	5 ± 35	9 ± 22	83 ± 19[cg]	56 ± 7[bdgk]
4) PTH-Mid G-5	24 ± 29[k]	−8 ± 35[dk]	52 ± 25[ahij]	53 ± 34[bfl]

Cuprophane vs. polyacrylonitrile, [a] $P < 0.05$, [b] $P < 0.005$, [c] $P < 0.0005$
Beginning vs. end, [d] $P < 0.05$, [e] $P < 0.005$
1 vs. 2, 3, 4, [f] $P < 0.05$, [g] $P < 0.005$, [h] $P < 0.0005$
2 vs. 3, 4, [i] $P < 0.05$
3 vs. 4, [j] $P < 0.005$
[k] $N = 7$
[l] $N = 5$

0.26) and end (Intact, −0.20 ± 0.38; Late C-52, 0.08 ± 0.20; Late C-97, 0.08 ± 0.18; Mid, −0.09 ± 0.30) of hemodialysis. With polyacrylonitrile, values are significantly higher at the beginning (Intact, 0.99 ± 0.27, $P < 0.005$; Late C-52, 0.63 ± 0.11, $P < 0.0005$; Late C-97, 0.66 ± 0.13, $P < 0.0005$; Mid, 0.42 ± 0.21, $P < 0.05$) and end (Intact, 0.72 ± 0.12, $P < 0.0005$; Late C-52, 0.51 ± 0.12, $P < 0.0005$; Late C-97, 0.46 ± 0.08, $P < 0.0005$; Mid, 0.41 ± 0.25, $P < 0.05$) of hemodialysis. Differences between the beginning and end of hemodialysis, in the various iPTH assays are similar to those described for the iPTH clearances.

Furthermore, when the gel chromatography profile of the plasma iPTH entering and exiting each filter was analyzed with the three carboxylterminal antisera at the beginning of hemodialysis (Fig. 2), it could be seen that there was no quantitative alteration in the various iPTH peaks with the cuprophane membrane, when antisera C-52 and C-97 were used to measured iPTH, and only a small difference in the smaller molecular forms when antiserum G-5 was used. On the other hand, all molecular forms of iPTH measured with the three different antisera were less when passing through the polyacrylonitrile membrane, explaining the clearance data.

Data obtained during in vitro dialysis of ^{131}I-bPTH(1-84) and ^{125}I-bPTH(41-84) are shown in Table 4. The clearance of ^{131}I-bPTH(1-84) was about 10 to 20 times higher with the polyacrylonitrile membrane than with the cuprophane membrane. ^{131}I-cpm recovered in the ultrafiltrate explained less than 5% of the clearance with the polyacrylonitrile membrane and less than 15% with the other membrane. The clearance of ^{125}I-bPTH(41-84) was also 10 to 20 times higher with the polyacrylonitrile membrane. ^{125}I-cpm in the ultrafiltrate explained at the maximum 25% of the clearance phenomenon with this membrane, but most of the clearance with the cuprophane membrane, although a large variation was observed in relation with the very low clearance values. The amount of radioactivity present on 1 g of fibers coming from the two different filters was 76451 ± 2059 cpm of ^{125}Iodine and 51656 ± 5599 of ^{131}Iodine for the polyacrylonitrile filter ($N = 2$), and only 9517 ± 2750 cpm of ^{125}Iodine and 7580 ± 849 cpm of ^{131}Iodine for the cuprophane filter ($N = 2$). The ratio of ^{131}I/^{125}I was 0.40 ± 0.03 in the serum used for in vitro dialysis, and 0.68 ± 0.05 and 0.82 ± 0.15 in the fibers coming from the polyacrylonitrile filter and the cuprophane filter, respectively, which is in accordance with the greater adherence of ^{131}I-bPTH(1-84) suggested by the preceeding data.

Discussion

This study was designed to outline factors that could affect plasma iPTH concentration during hemodialysis. Existing literature suggested that both dialysate Ca^{++} concentration [3, 5, 8, 12] dialysis membrane [14] and also the type of iPTH immunoassay used [3, 5, 8, 12] could influence the measurement of plasma iPTH during hemodialysis. To control variables related to these factors, we used a dialysate Ca^{++} concentration of 1.37 mmol/liter which usually increases plasma Ca^{++} concentration by 0.06 mmol/liter during four hours of dialysis and four different PTH immunoassays, which together were able to recognize most of the molecular forms of iPTH so far described in circulation, including intact hormone or hPTH(1-84), large late carboxylterminal fragments [17, 20], smaller late carboxylterminal fragments [17] as well as large and small mid carboxylterminal fragments [19]. We also selected a classical cuprophane membrane and a polyacrylonitrile membrane, more permeable to middle molecules, for comparison purposes.

Both membranes were equally effective in reducing BUN and creatinine and the extracorporeal clearance of these substances was similar. Even though hemoconcentration was more marked with the polyacrylonitrile filter, the level of ionized Ca^{++} achieved at the end of hemodialysis was similar with both filters. During dialysis with a cuprophane membrane, the increase in ionized Ca^{++} only influenced the concentration of intact iPTH, which decreased by 50%. Since the clearance of intact hormone or of various fragments measured by the different assays through the cuprophane filter was minimal, we conclude that a decreased secretion of intact hormone by the parathyroid glands as well as a persistent endogenous hepatic clearance of intact hormone in face of renal failure [21, 22] contributed to the lower iPTH concentration. The fact that the amount of hormone measured by various carboxylterminal assays was not affected by hypercalcemia despite a decreased production of intact hormone reflects the longer half-life of these fragments in renal failure, the major role played by the kidney in their metabolic clearance, and the lower capacity of other tissues to clear these fragments [16]. The only exception in some of our patients was a small extracorporeal clearance of mid carboxylterminal iPTH in particular in the patient in whom the gel chromatography profile of iPTH entering and exiting the filter was performed at the beginning of hemodialysis.

Although some other studies [11, 13] have clearly demonstrated permeability of the cuprophane membrane to small amino or carboxylterminal fragments, and a concomitant decrease in circulation during hemodialysis, the overall influence on circulating iPTH has been regarded as negligible or related to the increase in serum Ca^{++} concentration during hemodialysis [3, 5, 8, 10]. This is particularly true when concentrations of Ca^{++} higher than 1.75 mmol/liter were used in the dialysate with a predominantly aminoterminal antiserum [3, 8]. Lower concentrations of Ca^{++} in the dialysate and carboxylterminal directed antisera resulted in virtually unchanged iPTH levels [3–7, 14].

Very different results were obtained with the polyacryloni-

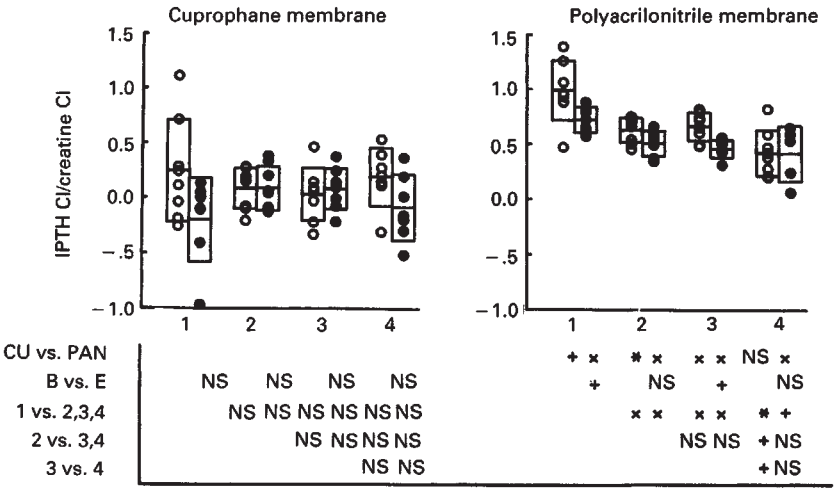

Fig. 1. *The ratio of iPTH clearance/creatinine clearance is illustrated for the four iPTH assays used, at the beginning (B, ○) and end (E, ●) of hemodialysis.* Boxes represent mean ± SD. Statistical significance: +, $P < 0.05$; x, $P < 0.005$; *, $P < 0.0005$.

trile membrane. Despite a similar increase in ionized Ca^{++} concentration, we observed more than 50% decrease in iPTH concentrations measured with the four iPTH assays during hemodialysis. Furthermore, we observed a significant iPTH clearance through the filter with all the assays. Contrary to what we had initially expected, clearance was more marked for intact iPTH than for large fragments, and the same held true for large versus smaller fragments. This indicates that physicochemical properties of the membrane other than permeability to a given molecular weight were involved in iPTH clearance. In fact, the clearances appeared directly related to the degree of hydrophobicity of the iPTH molecules as outlined during HPLC separation of the various molecular forms of iPTH found in circulation [23]. This suggests that iPTH adhered to the filter. This hypothesis was reinforced by the fact that the clearance of intact iPTH or of late carboxylterminal fragments decreased at the end of hemodialysis, while that of smaller mid carboxylterminal fragments was less affected. This indicated that these fragments may in part be cleared due to the greater permeability of the polyacrylonitrile filter. Furthermore, even if the clearance of intact iPTH was equal to the creatinine clearance with the polyacrylonitrile membrane, the iPTH level measured at the end of dialysis was only 25% lower than the level obtained with the cuprophane membrane at a similar serum ionized calcium. This indicated that extracorporeal clearance only explained part of our results while decreased intact hormone secretion and continuous endogenous hepatic clearance contributed to the rest. Our results also demonstrated that extracorporeal clearance, through selection of an appropriate filter, is an effective way of acutely decreasing carboxylterminal fragments of iPTH in renal failure.

Experiments with in vitro clearances of ^{131}I-bPTH(1-84) and ^{125}I-bPTH(41-84) helped to determine precisely the role of ultrafiltration and adhesion in the clearance of iPTH. In vitro clearance of both tracers with the polyacrylonitrile membrane was 10 to 12 times higher than with the cuprophane membrane, in accord with the in vivo data. Furthermore, the amount of radioactivity recovered in the ultrafiltrate only explained 5% of ^{131}I-bPTH(1-84) and 20% of ^{125}I-bPTH(41-84) clearances, adhesion explaining the remaining 95% and 80%, respectively. Even

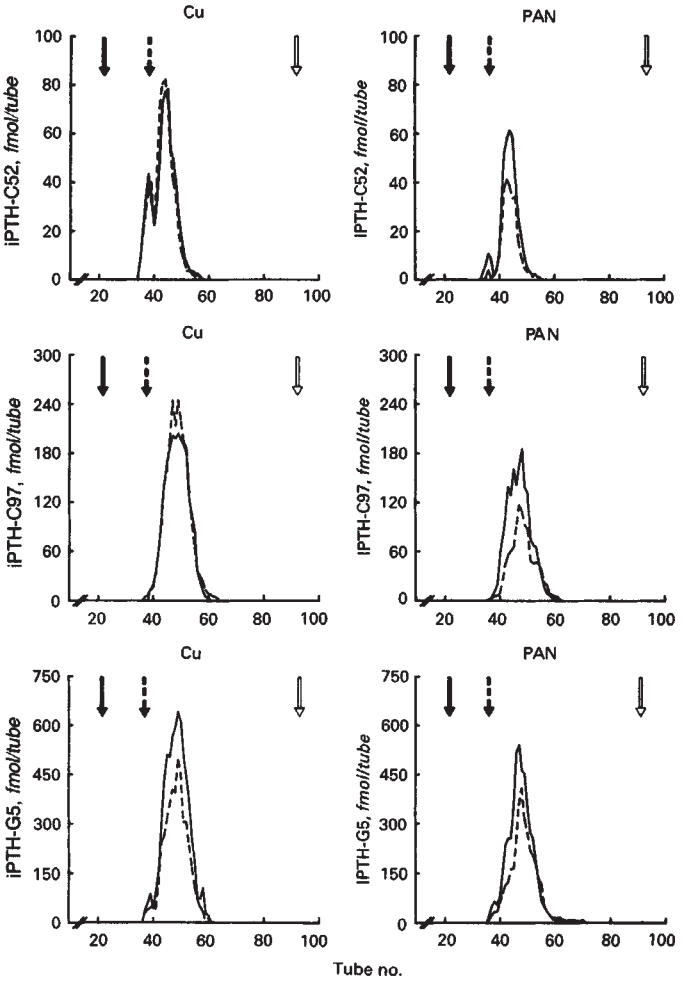

Fig. 2. *The gel chromatography profile of plasma iPTH entering (——) and exiting (– – –) the extracorporeal dialysis unit is illustrated for the same patient during two dialysis sessions, the first with a cuprophane (Cu) membrane and the second with a polyacrylonitrile (PAN) membrane.* Samples were obtained at the beginning of dialysis. Analysis of iPTH was performed with three different antisera. The position of elution of the void volume (⇩) of intact ^{125}I-bPTH(1-84) (⇩) and ^{125}Iodine (⇩) are indicated by arrows.

Table 4. Influence of dialysis filter on ^{131}I-bPTH(1-84) and ^{125}I-bPTH(41-84) clearance in vitro

Filter	Filtration pressure *mm Hg*	^{131}I-bPTH (1-84) clearance *ml/min*	^{131}I-cpm in ultrafiltrate *% of clearance*	^{125}I-bPTH (41-84) clearance *ml/min*	^{125}I-cpm in ultrafiltrate *% of clearance*
	50	35 ± 14	1 ± 1	32 ± 15	2 ± 2
Poly-	100	36 ± 15	2 ± 1	33 ± 13	8 ± 2
acrylonitrile	200	44 ± 12	4 ± 1	41 ± 11	19 ± 4
(N = 3)	275	49 ± 11	5 ± 2	49 ± 13	30 ± 7
	Σ	41 ± 13[b]	3 ± 2[a]	38 ± 13[b]	15 ± 12[a]
Cuprophane	200	2 ± 2	14 ± 13	2 ± 2	87 ± 63
(N = 3)	275	4 ± 0.1	19 ± 19	2 ± 2	73 ± 57
	Σ	3 ± 1	16 ± 13	2 ± 2	81 ± 53

Pump flow = ml/min

Σ polyacrylonitrile vs. Σ cuprophane, [a] $P < 0.005$; [b] < 0.0005

if clearances were much smaller with the cuprophane membrane, adhesion to the filter explained 85% of intact PTH clearance but only 25% of big carboxylterminal fragment clearance. In accord with this, 8 to 10 times more radioactivity was recovered on fibers coming from the polyacrylonitrile filter and the ratio of ^{131}I/^{125}I on these fibers also increased, reflecting the greater clearance and adherence of ^{131}I-bPTH(1-84) compared to ^{125}I-bPTH(41-84). This was even more true of the cuprophane fibers, since in this last case most ^{131}I-bPTH(1-84) adhered to the filter while little of ^{125}I-bPTH(41-84) did the same.

To our knowledge, this is the first detailed report demonstrating that the physicochemical properties of dialysis membranes have a substantial effect on the concentration of circulating molecular forms of PTH during hemodialysis. Fouchald, Gautvik and Gautvik [12], however, studied PTH movements across cellulose acetate, and demonstrated the presence of all the circulating molecular forms of PTH in the ultrafiltrate, but they only recorded a 10% decrease in circulating iPTH during four hours of hemodialysis with an iPTH clearance of less than 12 ml/min. Gueris et al [14] compared the influence of dialysis with a cuprophane membrane and a polyacrylonitrile membrane on serum carboxylterminal iPTH concentration and demonstrated a 57% decrease with the latter membrane and none with the former. They also explained their results via a greater permeability of the polyacrylonitrile membrane, a hypothesis which is refuted by our results. Furthermore, we have extended their findings to all molecular forms of circulating iPTH.

The practical consequences of these findings can be analyzed at various levels. First, iPTH measurements in hemodialyzed patients will have to be performed taking into account variables such as time since last dialysis, Ca^{++} concentration in the dialysate, filter characteristics, and also the type of iPTH assay selected. Since iPTH levels revert to predialysis levels, using either filter [14] between two dialysis sessions, predialysis iPTH concentration appears the most adequate single value to evaluate their parathyroid function. Also, the intact hormone assay appears the most appropriate assay to study hormone secretion under the influence of serum calcium concentration in these patients, since other molecular forms of iPTH are little affected, probably because of the very large peripheral pool in absence of renal clearance [16]. On the other hand, carboxylterminal assays of iPTH could become useful tools to evaluate the extracorporeal clearance of middle molecules by different filters, etc.

There is no doubt that plasma iPTH concentration reverts to the previous level between two hemodialysis sessions in this study as well as in a preceeding one using the same type of filters [14]. In the latter study, hemodialysis with a polyacrylonitrile membrane for three weeks also did not influence the basal iPTH concentration and thus the secondary hyperparathyroidism of these patients, as previously suggested by studies covering Ca^{++} concentration in the dialysate [3–8]. On the other hand, hemodialyzed patients in general would be subjected to less carboxylterminal iPTH and/or less middle molecules [24] in between dialysis sessions, possibly reducing their toxic effects [25, 26]. Although carboxylterminal fragments of iPTH have previously been considered inert molecules, recent data suggest that they may be mitogenic in vitro [27] and may influence osteoblast-like cells in given circumstances [28]. The relevance of these studies to renal failure is, for the moment, purely speculative, but nonetheless our findings offer a possible way of intervention.

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